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3-Deazaadenosine prevents atherosclerosis and graft vasculopathy

Description

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The invention relates to the use of 3-deazaadenosine (c^3Ado) for the preparation of a medicament against vascular diseases or graft rejection, especially atherosclerosis and graft vasculopathy.

- 10 Adhesion of leukocytes to the endothelial cell layer and their subsequent migration into the vascular wall are believed to play a key role during the development of atherosclerotic lesions. Monocytes/macrophages and lymphocytes, for example, are ubiquitous at all stages of atherosclerotic plaque development and propagate the local inflammatory process.
- 15 Furthermore, lipid-laden macrophages accumulate within the plaque, thereby leading to instability with consequent rupture, thrombosis and acute vessel closure (1-3).

- It has been widely appreciated that the inhibition of leukocyte adhesion and
- 20 migration may have protective effects on plaque development (4-6). Cellular adhesion and migration are mediated by various molecules of the selectin, integrin, and immunoglobulin superfamily, such as the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Previous studies have demonstrated increased
- 25 expression of VCAM-1 and ICAM-1 on the surface of endothelial and vascular smooth muscle cells of human plaques and in experimental models of atherosclerosis (7-13). A recent study on C57/BL6 mice with homozygous mutations for various adhesion molecules indicated that endothelial adhesion molecules are directly involved in the pathogenesis of
- 30 atherosclerosis (14).

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Adhesion of leukocytes to the vascular endothelium is also an early step in graft rejection, leading to the migration of inflammatory cells into underlying tissues. Endothelial cells contribute to adhesion by expressing several inducible cell surface molecules that bind various inflammatory cells. Together with MHC molecules, the adhesion molecules have an important role in T-cell activation. For inflammatory cell adhesion to activated endothelial cells at least three separate receptor/ligand pairs are involved: ICAM-1/LFA-1, VCAM-1/VLA-4, and E-selectin/sialyl Lewis X, and/or related carbohydrates on leukocytes.

Current models propose that members of the selectin gene family (E-, P-, and L-selectin) mediate the initial adhesive interactions, including leukocyte rolling, and that subsequent firm adhesion and diapedesis require activation-dependent engagement of integrins with their endothelial ligands and PECAM-1, respectively.

Several previous studies showed that VCAM-1, ICAM-1, P-selectin, and LFA-1 are extensively expressed on endothelial cells in rat heart allograft atherosclerosis. Treatment of animals with antibodies to VCAM-1, VLA-4, ICAM-1 and LFA-1 induced immunosuppression and inhibited graft atherosclerosis. Also the administration of ICAM-1 antisense oligonucleotides led to immunosuppression. In contrast, in ICAM-deficient mice no prolongation of cardiac allograft survival could be detected, indicating that this protein is not alone responsible for the induction of rejection or graft vasculopathy.

3-Deazaadenosine (c^3 Ado), a structural analog of adenosine, is an anti-inflammatory drug that has been shown to inhibit monocyte chemotaxis and phagocytosis (15-18). Furthermore, there are data revealing that this adenosine analog reduces tumor necrosis factor- α -induced macrophage adhesion to endothelial cells in vitro via the selective inhibition of ICAM-1 synthesis (19). The underlying molecular mechanisms have not yet been

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fully clarified. c^3 Ado inhibits cellular methylation of membrane phospholipids and suppresses adenosylhomocysteine hydrolase (20,21). However, it has been suggested that the biological actions of c^3 Ado are independent of these mechanisms (21-24).

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Because of its anti-inflammatory properties, c^3 Ado has been studied in a clinical trial in patients with rheumatoid arthritis (19,25), and this drug will be tested in humans for its in vitro antiviral (HIV) activity (26). The drug has never been tested in animal models of vascular disease.

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Surprisingly, it was found that c^3 Ado inhibits in vivo leukocyte adhesion and concomitant formation of atherosclerotic lesions via inhibition of the expression of endothelial cell adhesion molecules, such as VCAM-1 and ICAM-1, as demonstrated in female C57/BL6 mice. These animals are reproducibly susceptible to fatty lesion formation which closely resembles early atherosclerotic plaques detected in humans (27-31).

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Further, it was found that c^3 Ado inhibits graft rejection and graft atherosclerosis in transplanted hearts.

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An aspect of the present invention is the use of 3-deazaadenosine or analogs thereof for the manufacture of a medicament for the prevention and/or treatment of vascular diseases or graft rejection.

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A further aspect of the present invention is the use of 3-deazaadenosine and analogs thereof for the manufacture of a medicament for the prevention and/or treatment of diseases associated with the expression of adhesion molecules ICAM-1 and VCAM-1 in endothelial cells.

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According to the present invention 3-deazaadenosine and analogs thereof can be used. Examples of such analogs are salts of 3-deazaadenosine and precursors of 3-deazaadenosine which can be degraded in the body under

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physiological conditions to give 3-deazaadenosine, e.g. 3-deazaadenosine phosphates. Further examples of analogs are deazanucleosides such as 3-deazaadenosine-3'-monophosphoric acid, 3-deazaadenosine-3',5'-cyclophosphate and 3-deazaadenosine-5'-diphosphoric acid and salts and precursors thereof, respectively.

3-Deazaadenosine and analogs thereof are capable of preventing or delaying the occurrence of atherosclerosis. They prevent the appearance of fatty streak formation and all other stages of atherosclerotic plaque development in the intima of arterial blood vessels via inhibition of the expression of adhesion molecules in vascular endothelial cells. This leads to a reduction of adhesion and consecutive infiltration of monocytes into the subendothelial layer. Therefore the inhibition of the endothelial expression of VCAM-1 and ICAM-1 is the key mechanism, by which $c^3\text{Ado}$ inhibits atherosclerosis.

By the same mechanism $c^3\text{Ado}$ and analogs inhibit graft atherosclerosis in transplanted organs. Examples of transplanted organs are hearts, kidneys, livers, lung etc. The administration of 3-deazaadenosine and analogs thereof leads to reduced occlusion of blood vessels, particularly arterial blood vessels in transplanted organs. Furthermore, reduction of rejection of transplanted organs, for example, reduction of rejection of transplanted hearts is achieved.

Thus, $c^3\text{Ado}$ is suitable for the prevention and/or treatment of transplant vasculopathy.

Further preferred indications of 3-deazaadenosine and analogs thereof are prevention of rejection in xenotransplantation, prevention of restenosis after coronary interventions, especially after stent-implantation. Restenosis in stents (in-stent restenosis) can be prevented by coating the stent to be used with 3-deazaadenosine. The combination of covalent binding of 3-deaza-

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adenosine to stents is effective for the prevention of restenosis. Further preferred indications are prevention of reperfusion injuries, e.g. in the heart or in the lung, treatment and prevention of infectious and inflammatory coronary syndromes, prevention and treatment of dilated cardiomyopathy, prevention and treatment of viral myocarditis and prevention and treatment of infections by parasites such as Malaria tropica.

Another preferred indication is the use of 3-deazaadenosine and analogs thereof for reducing the level of homocysteine.

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3-Deazaadenosine and analogs thereof can be used individually or in combination with other medicaments. When treating vascular diseases, for example, combination with cholesterol-reducing agents is conceivable. To prevent graft rejection 3-deazaadenosine and analogs thereof can be used in combination with immunosuppressive medicaments, e.g. cyclosporine. 3-Deazaadenosine and analogs thereof also can be combined with azathioprine, cortisone, rapamycine, tacrolimus and other immunosuppressive drugs.

20 The medicament can be administered any way, e.g. parenterally by means of injection or orally. Oral administration, e.g. in the form of tablets, capsules etc. is preferred.

The dose administered depends on the kind and severity of the disease. Normally, the dose ranges between 0.1 to 500 mg/kg body weight per day, preferably between 1 to 200 mg/kg body weight per day. Administration can be effected over a short period of one or more days. However, administration is usually effected over a period of at least one week. The dose can be varied during the administration period, if necessary.

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A further aspect of the invention is a method of treating or preventing vascular diseases or graft rejection, comprising administering to a subject

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in need thereof, e.g. a human, an efficient amount of 3-deazaadenosine or an analog thereof.

Yet a further aspect of the invention is a method of treating or preventing diseases associated with expression of adhesion molecules ICAM-1 and VCAM-1 in endothelial cells comprising administering to a subject in need thereof, e.g. a human, an efficient amount of 3-deazaadenosine or an analog thereof.

The invention is explained in more detail by the following Figures and Examples.

Figure Legends

Fig.1 Morphometric analysis of C57BL/J6 aortic sections. Bar graphs showing average neointimal to medial area ratio (NI/M) for control mice (n=9), mice on atherogenic diet (n=9), and mice on atherogenic diet and treatment with 3-deazaadenosine (c^3 Ado) (n=9). Average NI/M was calculated for 3 histological sections per animal. Intimal/neointimal thickness as measured by NI/M was 94% lower in mice treated with c^3 Ado compared to mice fed atherogenic diet without c^3 Ado ($p < 0.001$). There were no differences in NI/M between control and mice treated with c^3 Ado. Values are means \pm SEM.

Fig.2 Light micrograph of cross section of the aortic wall taken from female C57BL/6J mice fed with an atherogenic diet for 9 weeks. Note large lipid-rich, macrophage-rich fatty streak. Oil red O and hemalaun; original magnification $\times 100$; bar = 5 μ m.

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Fig.3 Corresponding light micrographs of cross sections of the aortic
(A-C) wall (200 μ m cranial to aortic valve) taken from female
C57BL/6J mice. (A) Group 2 fed with the atherogenic diet for
9 weeks demonstrates proliferation of the aortic intima. In
contrast the neointima was absent in mice treated with 3-
deazaadenosine (B). (C) Controls. Oil red O and hemalaun. A-
C: original magnification x40; bar = 10 μ m.

Fig.4 Cross sections of the ascending aorta with strong ICAM-1-
(A-F) positive (A) and strong VCAM-1-positive (B) endothelial cells
in mice on atherogenic diet. In mice treated with 3-
deazaadenosine (C+D) as well as in controls (E+F), no
endothelial expression of ICAM-1 and VCAM-1 in the aorta
was detected. ICAM-1 and VCAM-1 staining with monoclonal
rat anti-mouse antibodies using the APAAP method and
counterstaining with hemalaun. A-F: original magnification
x100; bar = 5 μ m.

Fig.5 High-magnification view of the aortic wall with an
atherosclerotic intimal lesion and CD11b-positive monocyte
(group 2, atherogenic diet). CD11b staining with monoclonal
rat anti-mouse antibodies using the APAAP method and
counterstaining with hemalaun. Original magnification x100;
bar = 5 μ m.

Example 1

Prevention of Atherosclerosis

1.1 Materials and Methods

1.1.1 Animals, Atherogenic Diet and Experimental Design

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Six to eight week old female C57BL/J6 mice (Charles Rivers Wiga, Sulzfeld, Germany) with an average body weight of 20 g were randomly divided into three groups:

- 5 Group 1: control animals (n=9) maintained on a normal mouse diet (Altromin, Standard Diet; Lage, Germany).

Group 2: nine animals receiving an atherogenic diet based on normal mouse chow but differing in total fat content (10% vs. 5%), protein content
10 (15.4% vs. 22%) and cholesterol content (1% vs. 0%). The total energy was 3790 vs. 3000 kcal/kg.

Group 3: nine animals on an atherogenic diet as described above, and 3-deazaadenosine (c^3 Ado) (Southern Research, Birmingham, AL, USA) mixed
15 into the food at a final concentration of 0.04 mg/g, according to a daily oral dose of 10 mg/kg c^3 Ado per animal.

The average intake of food for each animal was 5.2 g per day. Food and water were replenished every 2 days, and the volumes consumed for each
20 cage were recorded. The mice were kept in accordance with standard animal care requirements and were maintained on a 12 hour light-dark cycle with autoclaved water in a temperature-controlled environment. All animals remained healthy during the experimental period. After 9 weeks, mice were
25 sacrificed by inhalation of trichlormethane. The heart and the ascending aorta were removed and rinsed with PBS DULBECCO'S (Life Technologies, Paisley, Scotland). The lower half of the heart was transected along a line between the tips of the atria to obtain a base for the emerging ascending aorta. This procedure allowed an exact vertical embedding of the aorta in Tissue-Tek (Miles, USA) for optimal plane cross sections. The sections were
30 frozen in liquid nitrogen and stored at -80°C until further study.

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1.1.2 Quantification of Atherosclerotic Lesions and Neointimal Proliferation

A modification of the method described by Paigen et al. (29) was used to evaluate aortic lesion formation. The frozen tissue blocks were placed on a cryotome, and 8 μ m serial sections of the ascending aorta were collected on coated glass slides until we were able to locate the most cranial portion of the aortic sinus by examining unstained sections. Once this section (No.1) was identified, the cranial 35 sections, covering 280 μ m of the ascending aorta, were placed on separate slides for further evaluation. Every fifth section of the first 280 μ m of the ascending aorta was stained with oil red O (Riedel de Haen, Germany) and counterstained with hemalaun (Merck, Darmstadt, Germany) and elastic van Gieson (Chroma-Gesellschaft, Schmid GmbH, Kӧngen, Germany). Lesion area as well as intimal and medial area were blindly determined using a video-computer aided microscopy planimetry system (Zeiss, Oberkochen, Germany; Video Camera 3 CCD, Sony; 40x lens magnification; IBAS-2 with IBAS Version 2.0 Standard, Kontron, Mӓnchen, Germany).

The number of lesions was counted as described previously by reviewing every fifth section. This procedure resulted in 40 μ m between each evaluated section. The number of lesions in each cross section was also counted. Size of the lesions was determined as length of lesion along the luminal aortic perimeter which was related to the total luminal aortic perimeter in the section.

The extent of neointimal proliferation was quantified by measuring the area (μ m²) of the neointima and the media in each ascending aorta from 3 sections (No.10, 20 and 30). In each section four sectors (at 0°, 90°, 180° and 270°) of the vessel wall were analyzed in a defined window of 63 μ m x 63 μ m to measure neointimal area enclosed by the endothelial layer and internal elastic lamina and external elastic lamina. Ratio of neointimal to medial area ratio (NI/M) was averaged for each animal.

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1.1.3 Immunohistochemical Analysis

Serial cryostat sections (8 μ m) from the following 3 parts of the ascending aorta were chosen: 8-32 μ m, 120-144 μ m and 240-264 μ m distal to the aortic sinus. The frozen sections were fixed in ice-cold acetone and dried for 10 minutes. Sections were then incubated for 10 minutes in a 1:1000 dilution of mouse serum (Sigma, St. Louis, Missouri, USA). After rinsing with RPMI 1640 (Life Technologies, Paisley, Scotland), the sections were incubated for 40 minutes at room temperature with a 1:100 dilution of a monoclonal rat anti-mouse antibody against ICAM-1 (CF54) or VCAM-1 (CD106, Dianova GmbH, Hamburg, Germany). Detection of monocytes/macrophages was performed using monoclonal rat anti-mouse antibody CD11b (MAC-1) (Serotec Ltd., Oxford, England).

After additional washing steps with TRIS buffer (USB, Cleveland, OH, USA) and incubation with a secondary antibody (AffiniPureMouse anti-rat IgG, Dianova, Germany) (1:400) for 10 minutes, followed by incubation with a linking antibody (Dualsystem-Brückenantikörper; Dianova, Hamburg, Germany) (1:600) for 10 minutes, the sections were incubated with an alkaline phosphatase anti-alkaline phosphatase complex (APAPP) (1:50; Dianova, Hamburg, Germany) for 30 minutes. The development of the sections was performed in neufuchsin developer solution. Sections were finally counterstained with hemalaun (Merck, Darmstadt, Germany) for 10 seconds. Control sections were treated with linking secondary antibody and APAAP complex only.

1.1.4 Quantification of Immunohistochemical Staining

The intensity of staining with ICAM-1 and VCAM-1 was scored from 1 to 4 as follows: score 1 = no staining, score 2 = weak staining, score 3 = moderate staining and score 4 = strong staining of the vascular cells. The number of CD11b-positive cells was counted in each section, and the

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localization of the cells was classified as adherent to the wall, or localized in the intima or in the media.

1.1.5 Determination of Total Plasma Cholesterol Levels

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Blood was collected via the tail vein from all mice after overnight fasting prior to initiation of the experiment, after 35 days feeding the different diets, and at time of death. Total plasma cholesterol levels were determined using the CHOD-PAP method (Boehringer Mannheim, Germany) as described previously (32).

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1.1.6 Statistical Analysis

Results were analyzed by two-way analysis of variance with repeated measures and one-way analysis of variance with pairwise contrasts by Scheffé. Differences in expression of ICAM-1, VCAM-1 and CD11b-positive monocytes were analyzed with the nonparametric Kruskal-Wallis one-way Anova. Values of neointimal area and NI/M ratio were averaged for all groups and differences were analyzed with Kruskal-Wallis one-way Anova as well. Data shown are means \pm SEM. All tests were performed with SPSS for Windows Version 6.1.3.

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1.2 Results

1.2.1 Cholesterol Levels and Manifestation of Atherosclerosis

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Mean cholesterol concentrations in the study groups on day 35 were: Group 1 (control) 92 ± 3 mg/dl, group 2 (atherogenic diet) 445 ± 10 mg/dl and group 3 (atherogenic diet and c^3 Ado) 410 ± 19 mg/dl. Table 1 summarizes the characteristics of the different groups. Note the further increase from day 35 to day 65 during c^3 Ado treatment (group 3), which was statistically

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significant ($p < 0.026$) and may be due to an interaction of 3-deazaadenosine with lipoprotein metabolism.

In order to examine the aorta for fatty streak formation, 7 frozen sections
5 of the ascending aorta per animal were quantified. Control mice did not
show any atherosclerotic changes, whereas animals of study group 2
showed multiple lipid-containing lesions covering the vessel wall of the
ascending aorta as demonstrated with oil red O staining (Figure 2). The
average number of lesions per animal in this group was 5.4 ± 1.6 (range
10 0-14), and the percentage of the covered aortic lining was $3.4 \pm 2.8\%$ (see
Table 3). In contrast animals of group 3 which received c^3Ado in addition
to the atherogenic diet had no detectable lesions in the sections studied.

Mice in group 2 showed markedly enhanced neointimal proliferation when
15 compared with animals of group 1 or group 3 (Figure 3). Planimetric
analysis of the intimal areas of the proximal ascending aorta demonstrated
a strong increase of the neointima ($450 \pm 775 \mu m^2$) when compared to
control mice ($160 \pm 38 \mu m^2$; $p < 0.001$). Treatment with c^3Ado in mice fed
the atherogenic diet resulted in an inhibition of neointimal proliferation
20 ($125 \pm 32 \mu m^2$; $p < 0.001$). Neointimal thickness as measured by NI/M ratio
was 94% lower in mice treated with c^3Ado as compared to in mice with
atherogenic diet alone (0.002 ± 0.0004 vs. 0.033 ± 0.005 ; $p < 0.001$).
Control mice showed the same NI/M ratio compared to the treatment group.
In Table 3 and Figure 1 these results are summarized.

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1.2.2 Monocytes and Adhesion Molecules ICAM-1 and VCAM-1

Animals fed the atherogenic diet showed CD11b-positive monocytic cells
adhering to the endothelium and located within the intima of the aorta.
30 Most of these cells were associated with atherosclerotic lesions (Figure 5).
In contrast, no monocytic cells within the intima and only a few adherent
CD11b-positive cells on the luminal surface were detected in animals of

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group 3 which had received c^3 Ado. In sections from control animals on standard diet no monocytes were present.

We furthermore investigated the regulation of adhesion molecules VCAM-1
5 and ICAM-1 which have both been suggested to be directly involved in
atherosclerotic lesion formation. Immunohistological staining demonstrated
intense endothelial expression of VCAM-1 and ICAM-1 in all analyzed frozen
sections of the ascending aorta of mice on an atherogenic diet. Both
adhesion molecules were abundantly expressed in lesions with extension
10 toward non-involved regions. In striking contrast, the expression of VCAM-1
and ICAM-1 was completely absent in mice treated with c^3 Ado as it was in
control animals (Figure 4). c^3 Ado totally inhibited endothelial expression of
both adhesion molecules. For a quantification of the images, refer to Table
2.

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Tables

Table 1. Body Weight Development and Total Cholesterol Levels

	Body weight day 0 (g)	Body weight day 65 (g)	Total cholesterol day 0 (mg/dl)	Total cholesterol day 35 (mg/dl)	Total cholesterol day 65 (mg/dl)
Group 1 (control) (n=9)	19.7±0.2	23.1±0.2	70±5	92±3	66±4
Group 2 (atherog. diet) (n=9)	19.3±0.3	25.8±0.2*	59±6	445±10*	434±11*
Group 3 (atherog. diet+c ³ Ado) (n=9)	19.6±0.2	25.3±0.2*	70±6	410±19*	475±10*
	NS		NS		

Values are expressed as means ± SEM. Differences between controls and groups 2 and 3 were statistically significant. * p < 0.001, analyzed by two-way analysis of variance with repeated measures and one-way analysis of variance with pairwise contrast by Scheffé. NS indicates not significant.

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Table 2. Endothelial Expression of VCAM-1, ICAM-1 and Quantification of Endothelial and Intimal Monocytes.

	VCAM-1 expression score	ICAM-1 expression score	Amount of intimal monocytes‡	Amount of intimal monocytes‡
Group 1 (control) (n=9)	1.1±0.1	1.0±0	0±0	0±0
Group 2 (atherog.diet)	3.3±0.2*	2.9±0.3*	3.6±0.7†	1.1±0.2†
Group 3 (atherog.diet + c ³ Ado) (n=9)	1.0±0	1.0±0	0.5±0.2	0±0

Differences between group 2 and group 3 were statistically significant. * p < 0.001 and † p = 0.004, analyzed by mean rank sums obtained with the Kruskal-Wallis test.

‡ mean ± SEM per section scored out of 3 defined sections per animal.

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Table 3. Quantification of Neointimal and Medial Areas, and Frequency of Fatty Streak Lesions

	Neointimal Area† (μm^2)	Medial Area† (μm^2)	NI/M Ratio	Number of lesions ‡
6				
10				
Group 1 (control) (n=9)	$0.16 \pm 0.04 \times 10^3$	$60.6 \pm 2.1 \times 10^3$	0.003 ± 0.0006	0 ± 0
15				
Group 2 (atherog. diet) (n=9)	$4.5 \pm 0.8 \times 10^3^*$	$135.2 \pm 5.8 \times 10^3^*$	0.033 ± 0.005	$5.4 \pm 1.6^*$
20				
Group 3 (atherog. diet+c ³ Ado) (n=9)	$0.13 \pm 0.03 \times 10^3$	$62.7 \pm 2.5 \times 10^3$	0.002 ± 0.0004	0 ± 0

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Differences were statistically relevant. * $p < 0.001$, analyzed by mean rank sums obtained with the Kruskal-Wallis test.

†mean \pm SEM determined out of 3 sections per animal.

‡mean \pm SEM calculated from 7 sections per animal.

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Example 2

Blocking of Heart Allograft Rejection and Heart Allograft Atherosclerosis

5 Materials and Methods

2.1.1 Animals and Experimental Design

3-Deazaadenosine was orally administered by mixing into the regular food
10 at a final concentration of 0.04 mg/g, according to a daily oral dose of 10
mg/kg c3Ado per animal, in addition to or in absence of cyclosporine (0.2
mg/kg body weight) to Lewis rats which received heterotopically
transplanted hearts from Fisher rats. For a third control group, only
cyclosporine was administered daily to the other allograft recipients. All
15 animals got a standard diet over the whole study period. Allografts were
harvested on day 120, cardiac graft beat was assessed by daily palpation,
and the complete cessation of the graft beat was interpreted as rejection.
All of the rats accepted cardiac allografts without any further treatment for
the duration of observation.

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2.1.2 Immunohistochemical Analysis

For immunohistochemical analysis of VCAM-1 and ICAM-1, two complete
transverse sections of the transplanted heart approximately 3 mm in
25 thickness were obtained and stored in optimum cutting temperature
compound. Serial sections were cut and dipped in cold acetone for 10
minutes. The staining procedures were performed as described previously.
The expression of the adhesion molecules was scored on the basis of
endothelial cells and smooth muscle cells of epicardial arteries and
30 intramyocardial arterioles. Analysis was done semiquantitatively by scoring
the intensity of the staining and counting of the number of stained cells.

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2.1.3 Definition of Graft Vasculopathy

Definition of graft vasculopathy was performed as follows: Blood vessels that had a well-defined smooth muscle cell layer and internal elastica lamina in the vascular wall were identified as traceable arteries. Every complete cross-section of the traceable arteries was calculated. The sections were reviewed by two independent reviewers masked to the status of the slides, and histologic changes were assessed semiquantitatively. The changes of intimal thickening were scored as mild (score 1, < 25% occlusion of the lumen) when the intima was readily discernible, or as moderate (score 2, 25-50% occlusion) to severe (score 3, > 50% occlusion).

2.2 Results

2.2.1 Graft Survival and Complications

The isografts and allografts in rats that received 3-deazaadenosine or cyclosporine or both drugs together kept beating for the duration of observation. Strength of the allograft beat was comparable to that of isografts. No complications occurred as a result of 3-deaza therapy. In mice receiving 3-deaza grafts were completely free from rejection whereas animals receiving cyclosporine alone mild rejection was seen.

Therefore, 3-deazaadenosine significantly reduced rejection of transplanted hearts with the same (or better) efficacy as the known immunosuppressive drug cyclosporine.

2.2.2 Graft Atherosclerosis

Native hearts and isografts showed no intimal thickening in coronary arteries. In the group of rats that received cyclosporine the intramyocardial and epicardial arteries showed significant intimal thickening and one third

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of the vessels were nearly occluded after 120 days. In the group of rats that received 3-deazaadenosine there was almost no arterial intimal thickening.

- 5 Therefore, 3-deazaadenosine significantly inhibited graft atherosclerosis in the transplanted hearts.

2.2.3 Expression of Adhesion Molecules ICAM-1 and VCAM-1

- 10 Isografts and native hearts were equally free from ICAM-1 and VCAM-1 expression. In contrast, these molecules were extensively upregulated in the neointima of transplanted hearts receiving cyclosporine alone. Conversely, there was almost no expression of ICAM-1 and VCAM-1 in rats which received 3-deazaadenosine.

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Therefore, 3-deazaadenosine blocks the expression of ICAM-1 and VCAM-1 in the intima of coronary arteries from transplanted hearts.

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